SB761
U7R62
#32

United States
Department of
Agriculture

Timber,
Forest Pest,
& Cooperative
Forestry
Management

Forest Service
Denver, Colorado

Cold Stratification and Hydrogen Peroxide Seed Treatments

LIBRARY COPY
ROCKY MT. FOREST & RANGE
EXPERIMENT STATION

Effects of Cold Stratification and Hydrogen Peroxide Treatments on Seeds of Three Rocky Mountain Conifer Species

by

Lloyd R. Fuller

and

Diane M. Hildebrand

Plant Pathologists

Technical Report R2-32

Timber, Forest Pest and
Cooperative Forestry Management
Rocky Mountain Region
USDA Forest Service
11177 W. 8th Avenue
Lakewood, CO 80225

ABSTRACT

The effects of 3% or 6% hydrogen peroxide (H_2O_2), in conjunction with cold stratification, on seedcoat sterilization and seed germination of three Rocky Mountain conifer species were evaluated. Based on greatest germination, the recommended treatments for ponderosa pine (*Pinus ponderosa*) seed are stratification alone or stratification with soaking in 3% H_2O_2 for 8 hours. The recommended treatments for lodgepole pine (*Pinus contorta*) seed are stratification alone or stratification with soaking in 3% H_2O_2 for 4 hours, or no stratification and soaking in 3% H_2O_2 for 24 hours. The best treatment for Engelmann spruce (*Picea engelmannii*) seed was stratification alone because H_2O_2 severely depressed germination.

INTRODUCTION

Many fungi inhabit external and internal seed parts (Bloomberg, 1966; Harvey & Carpenter, 1975; Mungal & Sharma, 1975). Fortunately, most fungi associated with seeds are not virulent pathogens and do not adversely affect germination (Belcher and Waldrip, 1972). However, in container-grown trees, some weakly parasitic fungi may become a problem because of ideal disease-development conditions present in greenhouses.

Greenhouse managers have more flexibility in dealing with insect and disease pests than have managers of bareroot nurseries; however, excessive reliance on pesticides can result in otherwise-preventable problems and in the build-up of resistence in the pests. Fungicidal seedcoat dressings may control damage by seed-borne fungi (Hamilton & Jackson, 1951; Carlson & Belcher, 1969), but most fungicides tested are phytotoxic (Cayford & Waldron, 1967; Vaartaja, 1956) or reduce germination rates (Carlson & Belcher, 1969; Peterson, 1970; Urosevic, 1961).

Brief exposure of seeds to hydrogen peroxide (H_2O_2) sterilization has been proposed as an alternative to fungicidal seedcoat dressings (Trappe, 1961; Barnett, 1976; Pawuk, 1981). H_2O_2 also has been reported to stimulate germination of some western conifers (Ching & Parker, 1958). Barnett (1976) recommended the use of 30% H_2O_2 to control microorganisms of southern pine seeds; however, this concentration of H_2O_2 is extremely hazardous to handle. The following study was undertaken to determine if lower concentrations of H_2O_2 would provide acceptable control of seedcoat fungi on three Rocky Mountain conifers. The effects of cold stratification on germination and microfungi populations were also investigated.

MATERIALS AND METHODS

The study was designed to compare the effects of cold stratification, concentrations of H_2O_2, and treatment duration on seed germination and occurrence of seed-borne fungi. Seeds of three Rocky Mountain conifer species were obtained from the USDA Forest Service Mt. Sopris Tree Nursery, Carbondale, Colorado: ponderosa pine (Pinus ponderosa Laws) lot PIPO-04-05-000-075-65, lodgepole pine (Pinus contorta Dougl.) lot PICO-02-06-432-080-77, and Engelmann spruce (Picea engelmannii (Parry) Engelm.) lot PIEN 13-05-247-105-77.

Cold Stratification

Seeds of each species were divided into 2 groups to be stratified or unstratified. Seeds were soaked in sterile tap water for 24 hours at room temperature then either treated immediately (unstratified) with H_2O_2 or towel-dried and stored in zip-lock plastic bags at 2-4°C for 46-48 days (stratified) prior to treatment with H_2O_2.

H_2O_2 Treatments

Stratified and unstratified seed were treated with 0% (check), 3%, or 6% H_2O_2 for 4, 8, 24, or 48 hours. Treatments were staggered so that all treatment durations were completed at the same time (e.g. the 48 hour treatment was started 2 days prior to the 4 hour treatment). After treatment, seeds were rinsed with sterile distilled water. Seeds were handled aspetically following treatment.

Each combination of stratification, H_2O_2 concentration, and treatment duration consisted of approximately 30-35 seeds. For each tree species, treatments were replicated three times using separate batches of seeds and fresh H_2O_2. Replications were approximately 2 months apart.

Contaminants and Germination

To determine frequencies and genera of fungal contaminants following treatment, 10 seeds from each treatment combination were placed on 2% malt agar plates (2 plates of 5 seeds each) and incubated at room temperature for 5 days under constant fluorescent lighting.

To test germination following treatment, 10 seeds from each treatment combination were placed in 2 Petri plates (5 seeds each) on sterile, moistened filter paper. Each germination plate was enclosed within a zip-lock plastic bag to maintain high humidity, and incubated in a growth chamber (Sherer CEL 38-15) for 4 weeks under an 8 hour light period at 30°C and 16-hour dark period at 20°C. The chamber was reset for each replication.

Seed germination counts commenced at 7 days and continued at 3-4 day intervals for 4 weeks. Germinated seeds were tallied and removed from the plates. Seeds were considered germinated when the radicle was at least 3-times seed length and all structures appeared normal. After 4 weeks ungerminated seeds were cut open and examined to determine if they were healthy (but dormant or low in vigor) or diseased (i.e. soft, rotten, mummified, or otherwise unhealthy). Empty seeds were not counted.

The significance of the stratification and H_2O_2 treatment effects were determined separately for each species by factorial analysis of variance with three replications.

RESULTS

When seeds were hand sorted into bags (without debris and broken seeds) no mold grew on the seeds during stratification. Tables for each tree species are located in the Appendix (ponderosa pine, Tables 1-4; lodgepole pine, Tables 5-8' Engelmann spruce, Tables 9-12).

Ponderosa Pine Germination

Germination counts for ponderosa pine seed are summarized in Table 1. Significant differences in treatment effects on ponderosa pine seed germination are shown in Table 2. The effects of treatment duration were all significantly different; the overall effect of longer duration was decreased germination. Cold stratification resulted in significantly greater germination with fewer diseased seeds. H_2O_2 treatment resulted in significantly less germination and more diseased seeds. A significant interaction occurred between H_2O_2 concentration and cold stratification. Apparently, treatment of unstratified seed with H_2O_2 had a slight stimulatory effect, which, however, did not overcome the depressing effect of lack of stratification.

Ponderosa Pine Seedcoat Sterilization

The significant differences in treatment effects on ponderosa pine seedcoat sterilization are shown by analysis of variance (ANOVA) in Table 3. There was no significant effect due to treatment duration or cold stratification on ponderosa pine seed coat sterilization. H_2O_2 treatment significantly reduced seedcoat contamination; 6% H_2O_2 was more effective than 3%. The microorganisms isolated and their frequencies are presented in Table 4. Soaking stratified seed in 3% H_2O_2 for 8 hours resulted in less than 10% reduction in germination, and greatly reduced seedcoat contamination.

During the four-week germination period, only Alternaria sp. was noted as causing necrosis of radicles. Fusarium oxysporum, a causal agent of damping-off (Smith, 1975), occurred at low frequency but was not eliminated by 6% H_2O_2 treatment.

Lodgepole Pine Seed Germination

Germination counts for lodgepole pine seed are summarized in Table 5. Significant differences in treatment effects on lodgepole pine seed germination are shown by ANOVA in Table 6. Although treatment effects are significant, trends are confused by significant interactions. The greatest germination with the least diseased seed was achieved by stratification with no H_2O_2 treatment, stratification with 4 or 8 hours of soaking in 3% H_2O_2, stratification with 4 hours in 6% H_2O_2, or no stratification with 24 hours in 3% H_2O_2. Stratified seed was more susceptible to damage by longer duration and higher concentrations of H_2O_2, while germination of unstratified seed was stimulated by soaking 24 hours in 3% H_2O_2.

Lodgepole Pine Seedcoat Sterilization

Significant differences in treatment effects on sterilization of lodgepole pine seedcoats are shown by ANOVA in Table 7. Treatment with H_2O_2 significantly reduced lodgepole pine seedcoat contamination; 3% and 6% H_2O_2 treatment effects were similar.

Unstratified seed treated with H_2O_2 was significantly less contaminated than stratified seed. Cold stratification apparently encouraged fungal development and reduced the effectiveness of H_2O_2. Contamination in the stratified check and unstratified check batches were similar, resulting in a significant interaction between concentration and stratification. The main effects of concentration and stratification are strong, so the interaction is not of major concern (Snedecor and Cochran, 1973).

Shorter treatment durations resulted in significantly less contamination. This was especially true for stratified seed, where longer durations resulted in more diseased seed. The contaminants isolated and their frequencies are presented in Table 8.

Engelmann Spruce Seed Germination

Germination counts for Engelmann spruce seed are summarized in Table 9. Significant treatment effects on the germination of Engelmann spruce seed are shown by ANOVA in Table 10. Treatment with H_2O_2 significantly depressed germination, the effects of 3% and 6% H_2O_2 were similar. Overall, effects from cold stratification and treatment duration were not significant. The significant interaction between H_2O_2 concentration and stratification is due to the stratified check batches having better germination than all others, while unstratified check batches were similar to all H_2O_2-treated batches.

Engelmann Spruce Seedcoat Sterilization

Significant differences in treatment effects on Engelmann spruce seedcoat sterilization are shown by ANOVA in Table 11. Treatment with H_2O_2 significantly reduced seedcoat contamination; the effects of 3% and 6% H_2O_2 were similar. Duration of treatment had no significant effect. Unstratified seed treated with H_2O_2 was significantly less contaminated than stratified seed. The significant interaction between H_2O_2 concentration and stratification was due to high contamination in both the stratified and unstratified check batches. The microorganisms isolated from spruce seeds and their frequencies are listed in Table 12.

DISCUSSION

Hydrogen peroxide has been previously used to reduce contamination of seed and improve germination (Carter and Jones, 1962; Edwards and Sutherland, 1979; Trappe, 1961; Barnett, 1976; James and Genz, 1981). This study shows that seeds of different tree species respond differently to H_2O_2 treatment and stratification.

During the four weeks seeds were germinated, only Alternaria spp. were noted as causing necrosis of seedling radicles. This has also been reported by others (Urosevic, 1964; Prisyozhnyuk, 1960). The incidence of Alternaria spp. was reduced in lodgepole pine and Engelmann spruce seed and was eliminated in ponderosa pine seed by H_2O_2 treatment. Penicillium spp. comprise a considerable portion of the surface microflora of forest tree seeds; they are capable of reducing germination considerably, especially for dormant seed (Urosevic, 1961). Hydrogen peroxide treatments greatly reduced the incidence of Penicillium spp., but did not eliminate them. Fusarium spp., some of which are causal agents of pre- and post-emergence damping-off, occurred in low frequency in ponderosa pine seed, and were not eliminated by H_2O_2 treatment.

According to Barnett (1976), an effective seedcoat sterilant should eliminate all seedcoat microorganisms. This is not necessarily true. More important than the elimination of all fungi is whether microorganisms surviving the treatment are likely to cause disease. Use of 3% or 6% H_2O_2 did not eliminate all seedcoat fungi, but potential pathogens were consistently reduced. Many of the fungi (e.g. Aspergillus spp., Aureobasidium sp., and Penecillium spp.) are common seedcoat and soil inhabitants which at worst are weak pathogens and are usually saprophytic (Raper & Thom, 1949; Edwards & Sutherland, 1979; James & Genz, 1982). The surviving fungi could destroy individual seeds, but not pose a threat of disease outbreaks. Hydrogen peroxide treatment should certainly be considered if seed lots are known to be "dirty", or are to be grown in containers. The use of sterilized seed in bareroot operations would be less effective because of the microorganisms already present in the soil.

Cold stratification is a common nursery treatment for ponderosa pine and lodgepole pine seed (Krugman and Jenkinson, 1974). In this study stratification also resulted in optimum germination of ponderosa pine and lodgepole pine seed. Treatment with H_2O_2 depressed germination, but soaking stratified ponderosa pine seed in 3% H_2O_2 for 8 hours resulted in less than 10% reduction in germination, while reducing seedcoat contamination 95%. Soaking stratified lodgepole pine seed in 3% H_2O_2 for 4 hours depressed germination only slightly and reduced seedcoat contamination 65%. Unstratified lodgepole pine seed soaked in 3% H_2O_2 for 24 hours was stimulated to a germination percentage only slightly less than stratified check seed, and seedcoat contamination was reduced over 60%.

Although Engelmann spruce seed is usually not stratified in germination tests, the common nursery practice is to do so (Safford, 1974). In this study, stratified Engelmann spruce seed showed optimum germination. Treatment with H_2O_2 was very deleterious to this thin-coated seed, especially to stratified seed. If seedcoat sterilization is desired for Engelmann spruce seed, seed should be unstratified and soaked for 4 hours in 6% H_2O_2. This should result in 20% reduction in germination and 95% reduction in seedcoat contamination, compared to untreated stratified seed.

LITERATURE CITED

Barnett, J. P. 1976. Sterilizing southern pine seeds with hydrogen peroxide. Tree Planters' Notes 27(3):17-19.

Belcher, E. W., Jr. and B. T. Waldrip, Jr. 1972. Effect of thiram on seed mold and germination of slash pine seed. Proc. Assoc. Off. Seed Anal. 62:91-93.

Bloomberg, W. J. 1966. The occurrence of endophytic fungi in Douglas-fir seedlings and seed. Can. J. Bot. 44:413-420.

Carlson, L. W. and J. Belcher. 1969. Seed treatment fungicides for control of conifer damping-off: laboratory and greenhouse tests, 1967-68. Can. Plant Dis. Survey 49:33-42.

Carter, M. C. and L. Jones. 1962. The effect of hydrogen peroxide on the germination of loblolly and slash pine seed. USDA For. Serv. Southeast For. Exp. Sta., Paper 141, 12 p.

Cayford, J. H. and R. M. Waldron. 1967. Effects of captan on the germination of white spruce, jack and red pine seed. For. Chron. 43:381-384.

Ching, T. M. and M. C. Parker. 1958. Hydrogen peroxide for rapid viability tests of some coniferous tree seeds. For. Sci. 4:128-134.

Edwards, D. G. W., and J. R. Sutherland. 1979. Hydrogen peroxide treatment of Abies seeds. Canad. For. Serv. Bi-Monthly Res. Notes 35:3-4.

Hamilton, J. R. and L. W. R. Jackson. 1951. Treatment of shortleaf pine and loblolly pine seed with fungicidal dusts. Plant Dis. Rep. 35:274-276.

Harvey, G. M. and L. R. Carpenter. 1975. Fungi on stored Douglas-fir cones -- a problem? Tree Planters' Notes 26(4):16, 17, 24.

James, R. L. and D. Genz. 1981. Ponderosa pine seed treatments: Effects on seed germination and disease incidence. USDA For. Serv., Northern Region, Rep. No. 81-16, 13 p.

James. R. L. and D. Genz. 1982. Evaluation of fungal populations on ponderosa pine seed. USDA For. Serv., Northern Region Rep. No. 82-22, 21 p.

Krugman, S. L. and J. L. Jenkinson. 1974. Pinus L. Pine. p. 598-638 in: Schopmeyer, C. S., ed., Seeds of Woody Plants in the United States. USDA Agric. Handbook No. 450.

Mungal, R. L. and A. D. Sharma. 1975. Mycoflora of conifer seeds. Indian J. Mycol. Plant Pathol. (5):145-148.

Pawuk, W. H. 1981. Diseases of container-grown southern pine seedlings and their control. pages 47-50 in: Guldin, R. W., and J. P. Barnett, eds., Proc. of the Southern Containerized Forest Tree Seedling Conference, Aug. 1981.

Peterson, G. W. 1970. Seed-protectant chemicals affect germination of ponderosa pine seeds. Tree Planters' Notes 21(4):25-29.

Prisyozhnyuk, A. A. 1960. Fungus diseases of the seed and cones of conifers. IZV. Vyssh. Ucheb. Zaved. Les. ZH. 1:31-37. Cited in Mungal R. L., A. D. Sharma. 1975. Effect of seed mycoflora on pre-and post-emergence seedling rots of some important conifers in the Himachal Prodesh. Indian J. Mycol. Plant Pathol. 6:27-31.

Raper, K. B. and C. Thom. 1949. A Manual of the Penicillia. The Williams and Wilkins Co., Baltimore, 875 p.

Safford, L. O. 1974. Picea A. Dietr. Spruce. pages 587-597 in: Schopmeyer, C. S., ed., Seeds of Woody Plants in the United States. USDA Agric. Handbook No. 450.

Smith, R. S., Jr. 1975. Fusarium root disease. Pages 9-10 in: Peterson. G. W., and R. S. Smith, jr. eds., Forest Nursery Diseases in the United States. USDA For. Serv., Agric. Handbook No. 470.

Snedecor, G. W. and W. C. Cochran. 1974. Statistical Methods. The Iowa State Univ. Press. Ames, Iowa. 6th edition, p. 344.

Trappe, J. M. 1961. Strong hydrogen peroxide for sterilizing coats of tree seed and stimulating germination. J. For. 59:828-829.

Urosevic, B. 1961. The influence of saprophytic and semi-parasitic fungi on the germination of Norway spruce and Scots pine seeds. Proc. Int. Seed Test. Assoc. 26:537-556.

Urosevic, B. 1964. More important seed borne diseases of Czechoslovak forest trees. Proc. FAO/IUFRO Symposium of Internationally Dangerous Forest Diseases and Insects. Oxford, July 20-30, 1964, 5 p.

Vaartaja, O. 1956. Screening fungicides for controlling damping-off of tree seedlings. Phytopathology 46:387-390.

APPENDIX

PONDEROSA PINE
(Tables 1-4)

Table 1. Ponderosa pine seed germination results[1/].

Unstratified Duration	H_2O_2 Concentration[2/]						Over all Mean	
	0% (Check)		3%		6%			
	G	D	G	D	G	D	G	D
48 hour	2.3	5.0	2.7	5.7	2.7	4.7	2.6	5.1
24 hour	3.0	3.7	3.7	5.3	4.0	4.0	3.6	4.3
8 hour	4.3	3.7	3.0	4.0	3.0	5.0	3.4	4.2
4 hour	5.0	3.7	3.7	5.3	4.7	3.3	4.5	4.1
Mean	3.7	4.0	3.3	5.1	4.8	4.3	3.5	4.4
Stratified								
48 hour	5.3	4.0	4.7	5.0	2.0	7.3	4.0	5.4
24 hour	5.7	3.0	5.0	5.0	3.7	4.7	4.8	4.2
8 hour	7.0	3.0	6.0	4.0	5.0	4.3	6.0	3.8
4 hour	6.7	2.7	5.3	4.0	4.3	5.0	5.4	3.9
Mean	6.2	3.2	5.3	4.5	3.8	5.3	5.1	4.3

1/ Average counts for three replications of 10 seeds per treatment.

2/ G = germinated; D = disease, rotten, or abnormal.
Dormant or low vigor (but healthy) seeds were not included in means.

Table 2. Analysis of variance for treatment effects on ponderosa pine seed germination due to H_2O_2 concentration, treatment duration, and cold stratification.

Source	d.f.	S.S.	M.S.	F[1]
Replication	2	42.25		
Treatments	23	126.20	5.49	3.26 **
Duration (D)	3	36.70	12.23	7.28 **
4 vs (8+24+48)	1	14.01	14.01	8.34 **
8 vs (24+48)	1	12.68	12.68	7.55 **
24 vs 48	1	10.01	10.01	5.96 *
Concentration (C)	2	20.08	10.04	5.98 **
0 vs (3+6)	1	16.00	16.00	9.52 **
3 vs 6	1	4.08	4.08	2.43 n.s.
Stratification (S)	1	36.12	36.12	21.50 **
D x C	6	7.59	1.27	0.76 n.s.
D x S	3	8.27	2.76	1.64 n.s.
C x S	2	14.09	7.05	4.19 *
D x C x S	6	3.35	0.56	0.33 n.s.
Error	46	77.42	1.68	
TOTAL	71	245.87		

[1] n.s. = not significant
* = significant ($P < 0.05$)
** = highly significant ($P < 0.01$)

Table 3. Analysis of variance for treatment effects on ponderosa pine seed coat sterilization due to H_2O_2 concentration, treatment duration, and cold stratification.

Source	d.f.	S.S.	M.S.	F[1]
Replication	2	26.08	13.04	
Treatments	23	1218.21	52.97	14.80**
Duration (D)	3	3.82	1.27	0.36 n.s.
Concentration (C)	2	1170.75	585.37	163.60 **
0 vs (3+6)	1	1139.06	1139.06	318.35 **
3 vs 6	1	31.69	31.69	8.86 **
Stratification (S)	1	7.35	7.35	2.05 n.s.
D x C	6	9.14	1.52	0.43 n.s.
D x S	3	8.60	2.87	0.80 n.s.
C x S	2	6.19	3.09	0.87 n.s.
D x C x S	6	12.36	2.06	0.58 n.s.
Error	46	77.42	1.68	
TOTAL	71	1321.71		

[1] n.s. = not significant
* = significant ($P < 0.05$)
** = highly significant ($P < 0.01$)

Table 4: Microorganisms isolated onto malt agar from ponderosa pine seed and their frequencies in percentages by treatment.

SPECIES	H_2O_2 Concentration 0% Check	3%	6%
1. Alternaria sp.	41.7	0	0
2. Aspergillus sp.	29.2	8.3	0
3. Aureobasidium sp.	87.5	12.5	4.2
4. Bacteria	29.2	25.0	8.3
5. Cladosporium sp.	0	4.2	0
6. Fusarium oxysporum	0	0	8.3
7. Trichoderma sp.	29.2	4.2	0
8. Mucor/Rhizopus sp.	62.5	0	0
9. Penicillium sp.	87.5	20.8	8.3
10. Sterile	0	76.3	92.5

LODGEPOLE PINE

(Table 5-8)

Table 5. Lodgepole pine seed germination results[1/].

Unstratified Duration	H_2O_2 Concentration[2/]						Overall Mean	
	0% Check		3%		6%			
	G	D	G	D	G	D	G	D
48 hour	5.7	1.0	7.7	1.0	7.7	1.3	7.0	1.1
24 hour	7.3	0.3	9.3	0.3	8.0	0.7	8.2	0.4
8 hour	8.3	0	6.7	0.3	6.7	0.3	7.2	0.2
4 hour	8.3	0.3	6.7	0.7	7.7	0.3	7.6	0.4
Mean	7.4	0.4	7.6	0.6	7.5	0.7	7.5	0.5
Stratified								
48 hour	9.0	1.0	5.0	5.0	3.0	6.7	5.7	4.2
24 hour	8.0	2.0	7.7	2.3	4.0	5.7	6.6	3.3
8 hour	9.0	1.0	9.3	0.3	8.0	1.7	8.8	1.0
4 hour	10.0	0	9.7	0	9.0	0.7	9.6	0.2
Mean	9.0	1.0	7.9	1.9	6.0	3.7	7.6	2.2

1/ Average counts for three replicates of 10 seeds per treatment.

2/ G = germinated; D = diseased, rotten, or abnormal.
Dormant or low vigor (but healthy) seeds were not included in means.

Table 6. Analysis of variance for treatment effects on germination of lodgepole pine seed due to H_2O_2 concentration, treatment duration, and cold stratification.

Source	d.f.	S.S.	M.S.	F[1]
Replication	2	12.19	6.10	
Treatments	23	212.32	9.23	3.34 **
Duration (D)	3	48.93	16.31	5.90 **
4 vs (8+24+48)	1	23.34	23.34	8.44 **
8 vs (24+48)	1	15.56	15.56	5.63 *
24 vs 48	1	10.03	10.03	3.63 n.s.
Concentration (C)	2	26.69	13.35	4.84 *
0 vs (3+6)	1	14.69	14.69	5.32 *
3 vs 6	1	12.00	12.00	4.34 *
Stratification (S)	1	0.35	0.35	0.13 n.s.
D x C	6	13.50	2.25	0.81 n.s.
D x S	3	49.04	16.35	5.91 **
C x S	2	28.86	14.43	5.22 **
D x C x S	6	44.95	7.49	2.71 *
Error	46	127.14	2.76	
TOTAL	71	351.65		

[1] n.s. = not significant
* = significant ($P < 0.05$)
** = highly significant ($P < 0.01$)

Table 7. Analysis of variance for treatment effects on sterilization of lodgepole pine seedcoats due to H_2O_2 concentration, duration, and cold stratification.

Source	d.f.	S.S.	M.S.	F[1]
Replication	2	243.75	121.87	
Treatments	23	877.54	38.15	5.64 **
Duration (D)	3	79.04	26.35	3.89 **
4 vs (8+24+48)	1	24.67	24.67	3.64 **
8 vs (24+48)	1	45.37	45.37	6.70 **
24 vs 48	1	9.00	9.00	1.33 n.s.
Concentration (C)	2	518.08	259.04	38.26 **
0 vs (3+6)	1	517.56	517.56	76.45 **
3 vs 6	1	0.52	0.52	0.08 n.s.
Stratification (S)	1	120.12	120.12	17.74 **
D x C	6	50.25	8.37	1.24 n.s.
D x S	3	12.82	4.27	0.63 n.s.
C x S	2	86.08	43.04	6.36 **
D x C x S	6	11.14	1.86	0.27 n.s.
Error	46	311.59	6.77	
TOTAL	71	1432.88		

[1] n.s. = not significant
* = significant (P = 0.05)
** = highly significant (P = 0.01)

Table 8. Microorganisms isolated onto malt agar from lodgepole pine seed, and their frequencies in percentages by treatment.

Species	H_2O_2 Concentration		
	0% Check	3%	6%
1. Alternaria sp.	16.7	8.3	8.3
2. Aspergillus sp.	33.3	4.2	8.3
3. Aureobasidium sp.	33.3	25.0	29.2
4. Bacteria	62.5	37.5	25.0
5. Chaetomidium sp.	0.0	0.0	4.2
6. Cladosporium sp.	4.2	0.0	0.0
7. Trichoderma sp.	25.0	4.2	0.0
8. Mucor/Rhizopus	79.2	0.0	0.0
9. Paecilomyces sp.	4.2	0.0	0.0
10. Penicillium sp.	70.8	29.2	33.3
11. Sterile	2.5	60.4	58.3

ENGELMANN SPRUCE

(Tables 9-12)

Table 9. Engelmann spruce seed germination results [1/]

Duration Unstratified	H_2O_2 Concentrations [2/]						Overall Mean	
	0% Check		3%		6%			
	G	D	G	D	G	D	G	D
48 hour	4.7	4.7	3.7	5.0	5.0	4.3	4.4	4.7
24 hour	5.7	3.3	5.7	4.0	3.7	4.7	5.0	4.0
8 hour	4.7	5.0	3.3	5.0	6.3	3.0	4.8	4.3
4 hour	5.0	4.0	3.3	5.7	5.7	4.0	4.7	4.6
Mean	5.0	4.2	4.0	4.9	5.2	4.0	4.7	4.4
Stratified								
48 hour	5.3	4.3	3.7	5.3	2.3	7.0	3.8	5.6
24 hour	7.7	2.3	4.7	5.3	4.0	5.0	5.4	4.2
8 hour	6.7	3.3	4.3	5.0	5.0	3.3	5.3	3.9
4 hour	7.0	3.0	5.0	5.0	5.7	4.0	5.9	4.0
Mean	6.7	3.2	4.4	5.2	4.3	4.8	5.1	4.4

1/ Average counts for three replications of 10 seeds per treatment.

2/ G = germinated; D = disease, rotten, or abnormal
Dormant or low vigor (but healthy) seeds were not included in means.

Table 10. Analysis of variance for the treatment effects on germination of Engelmann spruce seed due to H_2O_2 concentration, treatment duration, and cold stratification.

Source	d.f.	S.S.	M.S.	F[1]
Replication	2	36.86	18.43	
Treatments	23	113.11	4.92	1.99 *
Duration (D)	3	17.00	5.67	2.29 n.s.
Concentration (C)	2	37.03	18.52	7.50 **
0 vs (3+6)	1	34.03	34.03	13.78 **
3 vs 6	1	3.00	3.00	1.21 n.s.
Stratification (S)	1	2.00	2.00	0.81 n.s.
D x C	6	23.42	3.90	1.58 n.s.
D x S	3	8.11	2.70	1.09 n.s.
C x S	2	17.58	8.79	3.56 *
D x C x S	6	7.97	1.33	0.54 n.s.
Error	46	113.81	2.47	
TOTAL	71	263.78		

[1] n.s. = not significant
* = significant ($P < 0.05$)
** = highly significant ($P < 0.01$)

Table 11. Analysis of variance for treatment effects on sterilization of Engelmann spruce seedcoats due to H_2O_2 concentration, treatment duration, and cold stratification.

Source	d.f.	S.S.	M.S.	F[1]
Replication	2	71.86	35.93	
Treatments	23	1023.77	44.51	7.51 **
Duration (D)	3	14.77	4.92	0.83 n.s.
Concentration (C)	2	811.02	405.51	68.38 **
0 vs (3+6)	1	807.51	807.51	136.17 **
3 vs 6	1	3.51	3.51	0.59 n.s.
Stratification (S)	1	84.49	84.49	14.25 **
D x C	6	17.98	3.00	0.51 n.s.
D x S	3	29.85	9.95	1.68 n.s.
C x S	2	49.10	24.55	4.14 *
D x C x S	6	16.56	2.76	0.47 n.s.
Error	46	272.81	5.93	
TOTAL	71	1368.44		

[1] n.s. = not significant
* = significant (P = 0.05)
** = highly significant (P = 0.01)

Table 12. Microorganisms isolated onto malt agar from Engelmann spruce seed, and their frequencies (in percentages) by treatment.

Species	H_2O_2 Concentrations		
	0% Check	3%	6%
1. Alternaria sp.	20.8	4.2	4.2
2. Aspergillus sp.	54.2	12.5	12.5
3. Aureobasidium sp.	16.7	0.0	8.3
4. Bacteria	91.7	37.5	29.2
5. Cladosporium sp.	4.2	0.0	0.0
6. Trichoderma sp.	37.5	0.0	0.0
7. Mucor/Rhizopus	62.5	0.0	0.0
8. Penicillium sp.	37.5	33.3	33.3
9. Sterile	0.4	68.8	74.2